In the Specification:

JD 7/18/08

Please replace the paragraph beginning at page 1, line 16, with the following:

--Anthrax toxin is a three-part toxin secreted by *Bacillus anthracis* consisting of protective antigen (PA, 83 kDa), lethal factor (LF, 90 kDa) and edema factor (EF, 89 kDa) (Smith, H., *et al.*, *J. Gen. Microbiol.*, 29:517-521 (1962); Leppla, S.H., *Sourcebook of bacterial protein toxins*, p. 277-302 (1991); Leppla, S.H., *Handb. Nat. Toxins*, 8:543-572 (1995)), which are individually non-toxic. The mechanism by which individual toxin components interact to cause toxicity was recently reviewed (Leppla, S.H., *Handb. Nat. Toxins*, 8:543-572 (1995)). Protective antigen, recognized as central, receptor-binding component, binds to an unidentified receptor (Escuyer, V., *et al.*, *Infect. Immun.*, 59:3381-3386 (1991)) and is cleaved at the sequence $RKKR_{167}$ (SEQ ID NO:1) by cell-surface furin or furin-like proteases (Klimpel, K.R., *et al.*, *Proc. Natl. Acad. Sci. USA*, 89:10277-10281 (1992); Molloy, S.S., *et al.*, *J. B. Chem.*, 267:16396-16402 (1992)) into two fragments: PA63, a 63 kDa C-terminal fragment, which remains receptor-bound; and PA20, a 20 kDa N-terminal fragment, which is released into the medium (Klimpel, K.R., *et al.*, *Mol. Microbiol.*, 13:1094-1100 (1994)). Dissociation of PA20 allows PA63 to form heptamer (Milne, J.C., *et al.*, *J. Biol. Chem.*, 269:20607-20612 (1994); Benson, E.L., *et al.*, *Biochemistry*, 37:3941-3948 (1998)) and also bind LF or EF (Leppla, S.H., *et al.*, *Bacterial protein toxins*, p. 111-112 (1988)). The resulting hetero-oligomeric complex is internalized by endocytosis (Gordon, V.M., *et al.*, *Infect. Immun.*, 56:1066-1069 (1988)), and acidification of the vesicle causes insertion of the PA63 heptamer into the endosomal membrane to produce a channel through which LF or EF translocate to the cytosol (Friedlander, A.M., *J. Biol. Chem.*, 261:7123-7126 (1986)), where LF and EF induce cytotoxic events.--

Please replace the paragraph beginning at page 2, line 31, with the following:

--The crystal structure of PA at 2.1 Å was solved by X-ray diffraction (PDB accession 1ACC) (Petosa, C., *et al.*, *Nature*, 385:833-838 (1997)). PA is a tall, flat molecule having four distinct domains that can be associated with functions previously defined by

biochemical analysis. Domain 1 (aa 1-258) contains two tightly bound calcium ions, and a large flexible loop (aa 162-175) that includes the sequence $RKKR_{167}$ (SEQ ID NO:1), which is cleaved by furin during proteolytic activation. Domain 2 (aa 259-487) contains several very long ß-strands and forms the core of the membrane-inserted channel. It is also has a large flexible loop (aa 303-319) implicated in membrane insertion. Domain 3 (aa 488-595) has no known function. Domain 4 (aa 596-735) is loosely associated with the other domains and is involved in receptor binding. For cleavage at $RKKR_{167}$ (SEQ ID NO:1) is absolutely required for the subsequent steps in toxin action, it would be of great interest to engineer it to the cleavage sequences of some disease-associated proteases, such as matrix metalloproteinases (MMPs) and proteases of the plasminogen activation system (e.g., t-PA, u-PA, etc., *see, e.g.,* Romer *et al., APMIS* 107:120-127 (1999)), which are typically overexpressed in tumors.--

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Please replace the paragraph beginning at page 6, line 24, with the following:

--In one embodiment, the cell overexpresses a matrix metalloproteinase. In another embodiment, the matrix metalloproteinase is selected from the group consisting of MMP-2 (gelatinase A), MMP-9 (gelatinase B) and membrane-type 1 MMP (MT1-MMP). In another embodiment, the matrix metalloproteinase-recognized cleavage site is selected from the group consisting of GPLGMLSQ (SEQ ID NO:2) and GPLGLWAQ (SEQ ID NO:3).--

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Please replace the paragraph beginning at page 6, line 29, with the following:

--In one embodiment, the cell overexpresses a plasminogen activator or a plasminogen activator receiptor. In another embodiment, the plasminogen activator is selected from the group consisting of t-PA (tissue-type plasminogen activator) and u-PA (urokinase-type plasminogen activator). In another embodiment, the plasminogen activator-recognized cleavage site is selected from the group consisting of PCPGRVVGG, PGSGRSA, PGSGKSA, and PQRGRSA (SEQ ID NOS:4-7, respectively).--

JD 7/18/08

Please replace the paragraph beginning at page 7, line 29, with the following:

--In one embodiment, the matrix metalloproteinase or a plasminogen activator-recognized cleavage site is selected from the group consisting of PCPGRVVGG, PGSGRSA, PGSGKSA, PQRGRSA, GPLGMLSQ and GPLGLWAQ (SEQ ID NOS:4-7, 2 and 3, respectively).--

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Please replace the paragraph beginning at page 7a, line 2, with the following:

--Fig. 1. Generation of PA mutants can be specifically processed by MMPs. (A). Schematic representation of MMP substrate PA mutants. The furin cleavage site RKKR (SEQ ID NO:1) was replaced with gelatinase favorite substrate sequences GPLGMLSQ (SEQ ID NO:2) in PA-L1 and GPLGLWAQ (SEQ ID NO:3) in PA-L2. The arrows show the cleavage sites of furin or MMPs as indicated. (B). Cleavage of PA-L1 by MMP-2, MMP-9 and soluble form furin. As described in Materials and Methods, PA-L1 was incubated with MMP-2, MMP-9 and furin, respectively, aliquots were withdrawn at the time points indicated, and the samples were analyzed by western blotting with the rabbit polyclonal antibody against PA. (C). Cleavage of PA-L2 by MMP-2, MMP-9 and soluble form furin. PA-L2 was treated as in B. (D). Cleavage of WT-PA by MMP-2, MMP-9 and soluble form furin. WT-PA was treated as in B.--

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Please replace the paragraph beginning at page 34, line 26, with the following:

--Overlap PCR was used to construct the PA mutants with the furin site replaced by MMP substrate octapeptide GPLGMLSQ (SEQ ID NO:2) in PA-L1 and GPLGLWAQ (SEQ ID NO:3) in PA-L2. Wild type PA (WT-PA) expression plasmid pYS5 (Singh, Y., *et al.*, *J Biol Chem*, 264:19103-19107 (1989)) was used as template. We used 5' primer F (AAAGGAGAACGTATATGA (SEQ ID NO:8), underlined are SD sequence and start codon of PA) and the phosphorylated primer R1 (pTGAGTTCGAAGATTTTTGTTTTAATTCTGG (SEQ ID NO:9), annealing to the sequence corresponding to P_{154}-S_{163}) to amplify the fragment N. We used the mutagenic phosphorylated primer H1 (pGGACCATTAGGAATGTGGAGTCAAAGTACAAGTGC TGGACCTACGGTTCCG (SEQ ID NO:10), encoding MMP substrate GPLGMLSQ (SEQ ID NO:2) and S_{168}-P_{176}) and reverse primer R2 ACGTTTATCTCTTATTAAAAT (SEQ ID NO:11), annealing to the sequence compassing I_{589}-R_{595}) to amplify the mutagenic fragment M1. We used a phosphorylated mutagenic primer H2 (pGGACCAT TAGGATTATGGGCACAAAGTACAAGTGCTGGACCTACGGTTCCG (SEQ ID NO:12), encoding MMP substrate GPLGLWAQ (SEQ ID NO:3) and S_{168}-P_{176}) to amplify mutagenic fragment M2. Then used primer F and R2 to amplify the ligation products of N and M1, N and M2, respectively, resulting in the mutagenic fragments L1 and L2, in which the coding sequence for furin site ($RKKR_{167}$; SEQ ID NO:1) were replaced by MMP substrate sequence GPLGMLSQ and GPLGLWAQ (SEQ ID NOS:2 and 3), respectively. The HindIII/PstI digests of L1 and L2, which included the mutation sites, were cloned between HindIII and PstI site of pYS5. The resulting expression plasmids were named pYS-PA-L1 and pYS-PA-L2, their expression products, the PA mutated proteins, were accordingly named PA-L1 and PA-L2.--

Please replace the paragraph (Table 2) beginning at page 43, line 28, with the following:

--Table 2: u-TP and t-PA cleavage sites

Substrate sequence	SEQ ID NO:	u-PA Kcat/Km	t-PA Kcat/Km	a-PA:t-PA selectivity
PCPGRVVGG	4	0.88	0.29	3.0
PGSGRSA	5	1200	60	20
PGSGKSA	6	193	1.6	121
PQRGRSA	7	45	850	0.005

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Please replace the paragraph beginning at page 44, line 16, with the following:

--A modified overlap PCR method was used to construct the mutated PA proteins in which the furin site is replaced by the uPA and tPA physiological substrate sequence PCPGRVVGG (SEQ ID NO:4) in PA-U1, uPA favorite sequences PGSGRSA (SEQ ID NO:5) and PGSGKSA (SEQ ID NO:6) in PA-U2 and PA-U3, respectively, tPA favorite sequence PQRGRSA (SEQ ID NO:7) in PA-U4. The PA expression plasmid pYS5 (Singh, Y., *et al.*, *J Biol Chem*, 264:19103-19107 (1989)) was used as template. A 5' primer F, AAAGGAGAACGTATATGA (SEQ ID NO:8) (Shine-Dalgarno and start codons are underlined), and the phosphorylated reverse primer R1, pTGGTGAGTTCGA AGATTTTTGTTTTAATTCTGG (SEQ ID NO:13) (the first three nucleotides encodes P, the others anneal to the sequence corresponding to P_{154}-S_{163}), were used to amplify a fragment designated "N". A mutagenic phosphorylated primer H1, pTGTCCAGGAAG AGTAGTTGGAGGAAGTACAAGTGCTGGACCTACGGTTCCAG (SEQ ID NO:14), encoding CPGRVVGG (SEQ ID NO:15) and S_{168}-P_{176}, and reverse primer R2, ACGTTTATCTCTTATTAAAAT (SEQ ID NO:11), annealing to the sequence encoding I_{589}-R_{595}, were used to amplify a mutagenic fragment "M1". A phosphorylated mutagenic primer H2, pGGAAGTGGAAGATCAGCAAGTACAAGTGCTGGACCTAC GGTTCCAG (SEQ ID

NO:16), encoding GSGRSA (SEQ ID NO:17) and S_{168}-P_{176}, and reverse primer R2 were used to amplify a mutagenic fragment "M2". A phosphorylated mutagenic primer H3, pGGAAGTGGAAAATCAGCAAGTACAAGTGCTGGACCTA CGGTTCCAG (SEQ ID NO:18), encoding GSGKSA (SEQ ID NO:19) and S_{168}-P_{176}, and reverse primer R2, were used to amplify a mutagenic fragment "M3". A phosphorylated mutagenic primer H4, pCAGAGAGGAAGATCAGCAAGTACAAGTG CTGGACCTACGGTTCCAG (SEQ ID NO:20), encoding QRGRSA (SEQ ID NO:21) and S_{168}-P_{176}, and reverse primer R2, were used to amplify a mutagenic fragment "M4". Primers F and R2 were used to amplify the ligated products of N + M1, N + M2, N + M3, and N + M4, respectively, resulting in the mutagenized fragments U1, U2, U3, and U4 in which the coding sequence for the furin site ($RKKR_{167}$; SEQ ID NO:1) is replaced by uPA or tPA substrate. The *Hind*III/*Pst*I digests of U1, U2, U3, and U4 were cloned between the *Hind*III and *Pst*I sites of pYS5. The resulting expression plasmids were named pYS-PA-U1, pYS-PA-U2, pYS-PA-U3, and pYS-PA-U4, and their expression products, the mutated PA proteins, were accordingly named PA-U1, PA-U2, PA-U3, and PA-U4. One expression plasmid encoded a mutant in which $RKKR_{167}$ (SEQ ID NO:1) is replaced by PGG, expected not to be cleaved by any protease. Its expression plasmid and expression product were named pYS-PA-U7 and PA-U7, respectively.--

Please replace the paragraph beginning at page 48, line 5, with the following:

--The crystal structure of PA shows that the furin site, $RKKR_{167}$ (SEQ ID NO:1), is in a surface-exposed, flexible loop composed of aa 162 to 175 (Petosa, C., *et al.*, *Nature*, 385:833-838 (1997)). Cleavage in this loop by furin or furin-like proteases is essential to toxicity. Mutated PA proteins were constructed in which the furin-sensitive sequence $RKKR_{167}$ (SEQ ID NO:1) is replaced by uPA or tPA substrate sequences. In mutated PA protein PA-U1, PCPGRVVGG (SEQ ID NO:4), a peptide from P5 to P4' in the physiological substrate plasminogen, was used to replace $RKKR_{167}$ (SEQ ID NO:1). In PA-U2, $RKKR_{167}$ (SEQ ID NO:1) was replaced by a peptide, PGSGRSA (SEQ ID NO:5), containing the consensus sequence SGRSA (SEQ ID NO:22) from P3 to P2', which was recently identified as the